1652
#7

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Art Unit: 1652
NIELSEN, et al.	Examiner: PAK, Y.
Serial No.: 09/743,419	Washington, D.C.
Filed: November 29, 2001	April 22, 2003
For: METABOLICALLY ENGINEERED MICROBIAL CELL COMPRISING A MODIFIED REDOX ACTIVITY	Docket No.: NIELSEN=5 Confirmation No.: 2282

REQUEST TO VACATE

Commissioner of Patents
Washington, D.C. 20231

S i r :

The restriction requirement mailed March 26, 2003, based on "claims 1-70", should be vacated as it is based on the wrong claims set.

This is a U.S. national stage of PCT/DK99/00398. The international application as filed contained 70 claims. However, in the course of international preliminary examination, the original 70 claim set was replaced with a 59 claim set. The January 10, 2001 transmittal letter enclosed a "Courtesy Copy of the International Preliminary Examination Report with annexes containing claims 1-59 to be substituted for the original claims for examination in this case". We simultaneously filed two preliminary amendments, a main one cancelling claims 2-59, and the supplemental one cancelling claim 1 and adding claims 60-79.

Hence, the office action should have been based on claims 60-79 of the supplemental preliminary amendment, not on claims 1-70 of the PCT application as filed internationally.

Copies of the IPE Report, the transmittal letter, and the two preliminary amendments are enclosed herewith.

Respectfully submitted,

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G:\ipc\u-z\WHBE\Nielsen5\pto request to vacate.wpd

FILED: January 10, 2001

APPLICANT(S): Jens NIELSEN et al.

DOCKET NO.: NIELSEN5

BASED ON: PCT/DK99/00398

THE PATENT AND TRADEMARK OFFICE STAMP HEREON ACKNOWLEDGES RECEIPT OF THE ABOVE-IDENTIFIED APPLICATION, INCLUDING THE FOLLOWING PAPERS:

- [X] FEES $ 990.00 (CH # PTO-2038)
- [X] PCT APPLICATION
  - [ ] TRANSMITTAL LETTER REQUEST
  - [X] FEE CALCULATION SHEET
  - [X] SPECIFICATION (pages)
  - [ ] DRAWINGS (sheets; figures)
  - [X] SEQUENCE LISTING [X] WITH DISK
  - [X] APPOINTMENT OF AGENT
  - [X] INVITATION TO CORRECT DEFECTS
  - [X] REQUEST FOR RECTIFICATION
  - [X] DEMAND FOR CHAPTER II
  - [ ] ARTICLE 34 AMENDMENTS
  - [X] RESPONSE TO WRITTEN OPINION
- [X] U.S. NATIONAL PHASE OF INTERNATIONAL APPLICATION
  - [X] TRANSMITTAL LETTER
  - [X] DECLARATION (pages)
  - [X] SMALL ENTITY STATEMENT(S) (pages)
  - [X] PRELIMINARY AMENDMENT
- [ ] OTHER

- [X] SUPPLEMENTAL PRELIMINARY AMENDMENT
- [X] INFORMATION DISCLOSURE STATEMENT (pages)
  - [X] FORM 1449 (pages)
  - [X] PATENTS AND/OR PRINTED PUBLICATIONS
- [X] SEQUENCE LISTING [X] WITH DISK
- [X] COURTESY COPY:
  - [X] SPECIFICATION (84 pages)
  - [X] DRAWINGS (6 sheets; 1-5 figures)
  - [X] 1ST PAGE INTERNATIONAL PUBLICATION
  - [X] INTERNATIONAL SEARCH REPORT
  - [X] IPER [X] WITH [ ] WITHOUT ANNEXES
  - [X] ENGLISH LANGUAGE TRANS OF SPECIFICATION AS FILED
  - [X] ENGLISH LANGUAGE TRANS OF ANNEXES TO IPER
  - [X] SUBSTITUTE SPECIFICATION

B&N-5

INITIALS: WRP

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
29945JL		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK99/00398	12/07/1999	10/07/1998
International Patent Classification (IPC) or national classification and IPC C12N15/52		
Applicant NIELSEN, Jens et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04/02/2000	30.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer van Heusden, M Telephone No. +49 89 2399 8145

Form PCT/IPEA/409 (cover sheet) (January 1994)

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-74	as originally filed	

Claims, No.:

1-59	with telefax of	03/11/2000

Drawings, sheets:

1/6-6/6	as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	18-19, 23-28, 32, 42-47, 49, 51-53, 58-59
	No:	Claims	1-17, 20-22, 29-31, 33-41, 48, 50, 54-57
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-59
Industrial applicability (IA)	Yes:	Claims	1-59
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Additional remarks to section II:

The priority claimed in the present application is valid for the present set of claims. Therefore documents D12-D16 do not constitute prior art within the meaning of Rule 64.1 PCT.

Additional remarks to section V:

1. Citations

The documents mentioned in this IPER are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc.

2. Novelty (Article 33(2) PCT)

2.1 The present application relates to a eukaryotic microbial cell, more specifically the yeast *Saccharomyces cerevisiae*, that comprises a transhydrogenase activity encoded by *Azotobacter vinelandii*. As a result, glycerol production is increased.

2.2 None of documents D1, D2 and D4-D8 discloses a eukaryotic microbial cell comprising an intracellular transhydrogenase activity encoded by a nucleic acid operably linked to an expression signal not natively associated with the nucleic acid.

2.3 However, the present application does not satisfy the criterion set forth in Article 33(2) PCT in that the subject matter of claims 1-17, 20-22, 29-31, 33-41, 48, 50 and 54-57 is not novel in view of Document D3.

Document D3 discloses a eukaryotic microbial cell comprising an intracellular transhydrogenase encoded by a nucleic acid operably linked to an expression signal not natively associated with the nucleic acid. However, D3 does not disclose that the expression of said transhydrogenase activity results in an increased production of a metabolite, such as ethanol or glycerol. In fact D3 discloses that both ethanol production and growth rate are decreased.

Interestingly, the microbial cell provided by the present alleged invention also shows reduced ethanol production and growth rate (see Table 2 on p. 60). Glycerol production is not measured in D3 and it is not explicitly disclosed that the production of a metabolite is increased. However, since the microbial cell disclosed in D3 is identical to the eukaryotic microbial cell as defined in claim 1, the increased production of a metabolite is inherent. Thus D3 anticipates the subject matter of claim 1. If the applicant considers that the eukaryotic microbial cell according to claim 1 differs from the microbial cell disclosed in D3, then it appears that an essential technical feature (responsible for the difference between the two cells) is missing from claim 1.

Furthermore, D3 discloses the decreased production of ethanol ('second metabolite'). The microbial cell of D3, in which a heterologous pyridine nucleotide transhydrogenase is over expressed, will inherently have increased levels of coenzymes (NAD, NADP or FAD). Thus D3 anticipates the subject matter of claims 2-13 and 15.

The transhydrogenase as disclosed in D3 falls within the scope of 'a functionally equivalent activity capable of catalysing a transhydrogenase activity' . Thus D3 also anticipates the subject matter of claim 14.

The microbial cell of D3 harbours no endogenous transhydrogenase (Table 1 on p. 669). The transhydrogenase used in D3 is a membrane-bound transhydrogenase from *E. coli*. The microbial cell of D3 is inherently in a broth suitable for culturing or for starting a fermentation. Thus D3 also anticipates the subject matter of claims 16, 17 and 20-22.

The microbial cell of D3 is described to have a reduced growth rate and a retarded ability of fermentation (p. 670, l. 19-20). Thus it appears that said cell produces ethanol, even if at a reduced or slower rate. Therefore the microbial cell of D3 is still suitable for the production of ethanol. Furthermore, as argued above, the cell must inherently show an increased glycerol production (since the microbial cell of D3 is identical to the eukaryotic microbial cell as defined in claim 1) and is thus also suitable for the production of glycerol. Consequently the microbial cell is also suitable for use in a preparation of a drinkable, edible or organoleptic

product. Thus D3 also anticipates the subject matter of claims 29-31 and 33-41.

D3 further describes a method of cultivating a microbial cell according to claim 1 in a suitable growth medium under such conditions that said cell is producing a first metabolite (ethanol) (p. 665, l. 36-39). Thus D3 also anticipates the subject matter of claims 48 and 50.

Furthermore, D3 discloses a method of constructing a microbial cell comprising the steps of operably linking a nucleotide sequence encoding a transhydrogenase activity with an expression signal not natively associated with said nucleotide sequence, and introducing said sequence into a eukaryotic microbial cell (the yeast *S. cerevisiae*), wherein said expression signal results in expression of said operably linked nucleotide sequence. Thus D3 also anticipates the subject matter of claims 54-57.

3. Inventive step (Article 33(3) PCT)

3.1 The present application does not seem to satisfy the criterion set forth in Article 33(3) PCT because the subject matter of claims 18-19, 23-28, 32, 42-47, 49, 51-53 and 58-59 does not involve an inventive step in view of documents D1-D3.

3.2 The closest prior art to evaluate the inventiveness of claims 23-26 and 28 is document D2, which discloses the purification of pyridine transhydrogenase from *A. vinelandii* (p. 528, left column, paragraph 3). The purification of this enzyme is also acknowledged in the description (p. 54, l. 19-21) by reference to Voordouw *et al.*, 1980. Once an enzyme is known and even more so when its purification is disclosed in the prior art, no inventive skill is needed to isolate the DNA sequence encoding the enzyme, to deduce the encoded amino acid sequence or to provide the encoded enzyme by recombinant means.

3.3 To transform a eukaryotic microbial cell with the transhydrogenase of *A. vinelandii* does not involve an inventive step in view of document D1 and D3, in combination with document D2. Document D1 discloses the transformation of *E. coli* with the soluble pyrimidine nucleotide transhydrogenase from *Pseudomonas fluorescens*. D2 discloses the high level of similarity between said transhydrogenase from *P.*

fluorescens and that from *A. vinelandii*. Therefore it is obvious that the transhydrogenase from *P. fluorescens* can be replaced by that of *A. vinelandii*. D1 discloses that transformation of *E. coli* with a soluble transhydrogenase results in an altered balance/oxidation state of coenzymes such as NAD/NADP or analogs, which in turn results in improved biotransformation, inherently providing increased production of a metabolite. No inventive step is needed to apply this teaching of D1 (relating to bacteria) to yeast. This is even more so in view of the disclosure in D3 in which transformation of a yeast with a transhydrogenase is disclosed. Whereas D3 teaches that transformation with membrane-bound transhydrogenase from *E. coli* does not result in improved fermentation, D3 also states that this may be due to the fact that the transhydrogenase used is membrane-bound and that further suggests that better results may be obtained with water-soluble transhydrogenases (p. 670, last paragraph). The results disclosed in D1 further support this suggestion. Thus, in view of documents D1 and D3, in combination with D2, no inventive step is needed to provide the microbial cell designated TN4 (as defined in claim 14). Also the use of said cell in the production of metabolites or drinkable/edible products does not involve an inventive step. Thus also the subject matter of claims 27, 42-47, 49, 51-53 and 58 does not comply with Article 33(3) PCT.

3.4 Claims 18, 19, 32 and 59 do not include any additional matter that could render them inventive as such. These claims would be allowable only in combination with a novel and inventive main claim.

4. Industrial applicability (Article 33(4) PCT)

The subject matter of claims 1-59 is industrially applicable.

Additional remarks to section VI:

Document D17, with a publication date of 16.09.99, a filing date of 11.03.99 and a priority date of 11.03.98, does not constitute prior art within the meaning of Rule 64.1(b). However, it appears to disclose features of the present set of claims. No check has been made as to whether the priority of this prior application has been

validly claimed.

Additional remarks to section VIII:

The following major objections are raised under **Article 6 PCT** concerning the clarity of the claims:

1. The subject matter of claim 1 is characterized by a result to be achieved, i.e. increased production of a first metabolite. However, the essential technical features required to arrive at such increased production of a metabolite are lacking from claim 1 (see also above under item V.3.4). Thus claims 1-22 and 29-57 and 59 are considered to lack clarity.

2. The wording '**operably linked**' in claims 1-2 lacks clarity in that the IPEA does not understand the exact meaning of this wording. Normally this wording refers to a sequence encoding e.g. an enzyme, which sequence is linked to a control sequence (i.e. a promoter) that controls the expression of said sequence. It is not clear to the IPEA how an enzyme activity is 'operably linked' to the production of a metabolite. The wording 'operably linked' is not suitable to define clearly and unambiguously the scope of claims 1-2.

3. The subject matter of claim 17 lacks clarity in that it is not clear to the IPEA what is intended by the wording '**in a natural host organism**'. The same observation applies to the wording 'in a cytoplasm **of a natural host organism**' in claim 18. Furthermore, it is noted that the feature of a polypeptide being localized 'in a cytoplasm' is considered to encompass both membrane-bound and soluble forms of the polypeptide.

4. The subject matter of claims 23, 24 and 28 lacks clarity in that the wording 'or **part thereof**, including **functionally equivalent derivatives**' is not suitable to define clearly and unambiguously the scope of these claims. This wording is open to individual interpretation and thus ambiguous: for example the transhydrogenase of *Pseudomonas fluorescens*, as disclosed in D1, can be considered a derivative (through substitutions/deletions/insertions) being functionally equivalent to the

transhydrogenase represented by SEQ ID NO:1, and would thus anticipate the subject matter of claims 23-25 and 28.

5. The wording '**comparable isolated microbial cell**' in claim 52 lacks clarity in that it is not clear to the IPEA what is meant with this wording.

6. The applicant has shown that the expression of transhydrogenase from *A. vinelandii* in *S. cerevisiae* results in increased glycerol production: according to example 1 of the application, production of glycerol is increased in the TN4 transformant (Table 2 on p. 60), whereas the production of ethanol and the growth rate (biomass) of TN4 is reduced (similar to the results disclosed in D3!). However, the examples do not show any evidence for the increased production of any metabolite other than glycerol. In fact they show a reduced rate of e.g. ethanol production. Therefore it seems that the present set of claims covers a large area of subject matter that is not enabled by the application, for instance the generalisation to an increase of **any** first metabolite, and to a decrease of **any** second metabolite, as well as the generalisation to any intracellular transhydrogenase.

INTERNATIONAL SEARCH REPORT

Int. Jonal Application No
PCT/DK 99/00398

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/52 C12N15/53 C12N9/00 C12N9/02 C12N9/06
C12N1/19 C12P7/06 C12P7/20 //(C12N1/19,C12R1:865)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 18909 A (FRENCH CHRISTOPHER EDWARD ;UNIV CAMBRIDGE TECH (GB); BRUCE NEIL CH) 7 May 1998 (1998-05-07)	1,2,6, 8-12,14, 17-19, 28-30, 37,44, 45,47, 51,53, 54,56, 57, 64-66,70
Y	Note: 82.2% nt sequence identity of SEQ ID NO:1 with SEQ ID NO:1 in 1395 nt overlap, 84.1% aa sequence identity of SEQ ID NO:2 with SEQ ID NO:2 in 464 bp overlap. page 2, line 25 -page 3, line 5 page 3, line 7 -page 4, line 2 page 4, line 9-13 example 3 claims 1-8	13,35

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 January 2000	11/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van de Kamp, M

Form PCT/ISA/210 (second sheet) (July 1992)

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INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/DK 99/00398

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VOORDOUW G ET AL: "Why are two different types of pyridine nucleotide transhydrogenase found in living organisms?" EUROPEAN JOURNAL OF BIOCHEMISTRY, DE, BERLIN, vol. 131, no. 3, April 1983 (1983-04), pages 527-533, XP000853531 ISSN: 0014-2956 cited in the application	31-34,36
Y	abstract page 527 page 528, left-hand column, paragraphs 2,3 page 528, right-hand column, paragraph 4 -page 529, left-hand column, paragraph 1 figure 1	13,35
X	TANTIRUNGKIJ M ET AL: "Expression of Escherichia coli transhydrogenase genes in Saccharomyces cerevisiae" MICROB. UTIL. RENEWABLE RESOUR. (1996), VOLUME DATE 1995, vol. 9, pages 664-672, XP000866358 the whole document page 670, paragraph 3	1-12, 14-19, 28-30, 37,38, 41,42, 44-62, 64-68,70
X	EP 0 733 712 A (AJINOMOTO KK) 25 September 1996 (1996-09-25) cited in the application page 2, line 48-55 page 3, line 3-5,11-45 examples 1-4 claims 1-8	1,2,6, 8-12,19, 28-30, 37,44, 45, 47-49, 51,53, 54,56, 57, 64-66,70
X	WO 96 41888 A (INST NAT RECH AGRONOMIQUE IN ;DEQUIN SYLVIE (FR); BARRE PIERRE (FR)) 27 December 1996 (1996-12-27) the whole document example 2 claims 1,10	1-9,19, 28-30, 37,38, 42, 44-61, 65-68,70

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/DK 99/00398

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE VOS W M: "Metabolic engineering of sugar catabolism in lactic acid bacteria;" ANTONIE LEEUWENHOEK J. MICROBIOL., vol. 70, no. 2-4, October 1996 (1996-10), pages 223-242, XP000866282 the whole document page 225, left-hand column, paragraph 2 -page 231, left-hand column, paragraph 1 page 236, left-hand column, paragraph 2 -page 238, right-hand column, paragraph 1	1-6, 8-11,20, 23,25, 28-30, 37,39, 43-45, 47-49, 51,53, 54, 56-58, 63,65, 66,70
X	LOPEZ DE FELIPE, F. ET AL: "The role of NADH -oxidation in acetoin and diacetyl production from glucose in Lactococcus lactis subsp. lactis MG1363" FEMS MICROBIOLOGY LETTERS, vol. 156, no. 1, 1 November 1997 (1997-11-01), pages 15-19, XP000866569 abstract page 19, left-hand column, line 23-29	1-6, 8-11, 28-30, 37,39, 43-45, 47-49, 51,53, 54, 56-58, 63,65, 66,70
X	EP 0 462 674 A (GIST BROCADES NV) 27 December 1991 (1991-12-27) the whole document claims 18,19	1,6, 28-30, 37,40, 44,45, 47,48, 51,53, 54, 56-58, 65,66
A	VAN DIJKEN, J.P. ET AL: "Redox balances in the metabolism of sugars by yeasts." FEMS MICROBIOLOGY REVIEWS, vol. 32, no. 3-4, 1986, pages 199-224, XP000866552 the whole document page 199-203 page 217 page 219-222	1-19,37, 38,41, 42,44-62

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INTERNATIONAL SEARCH REPORT

Int. Ional Application No
PCT/DK 99/00398

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VAN RIEL N A W ET AL.: "A structured, minimal parameter model of the central nitrogen metabolism in Saccharomyces cerevisiae: the prediction of the behaviour of mutants" JOURNAL OF THEORETICAL BIOLOGY, vol. 191, no. 4, 21 April 1998 (1998-04-21), pages 397-414, XP000852821 abstract page 398; figure 1 page 404; figure 4 page 409, left-hand column, line 5-18 page 410, right-hand column, line 7-11 page 411, left-hand column, line 2-6 ---	20-27
A	AVENDANO A ET AL: "GDH3 encodes a glutamate dehydrogenase isozyme, a previously unrecognized route for glutamate biosynthesis in Sacharomyces cerevisiae" JOURNAL OF BACTERIOLOGY,US,WASHINGTON, DC, vol. 179, no. 17, September 1997 (1997-09), page 5594-5597 XP002085996 ISSN: 0021-9193 abstract ---	21,22, 24,26
P,X	DATABASE EMPRO 'Online! EMBL ID AF159108, AC AF159108, 29 June 1999 (1999-06-29) BOONSTRA B ET AL.: "Azotobacter vinelandii soluble pyridine nucleotide transhydrogenase (sth) gene, complete cds" XP002128388 Note: 99.6% nt sequence identity with SEQ ID NO:1 in 1395 nt overlap. the whole document ---	1,2,6, 8-15, 17-19, 28-37, 64-66,70
P,X	ANDERLUND M ET AL.: "Expression of the Escherichia coli pntA and pntB genes, encoding nicotinamide nucleotide transhydrogenase, in Saccharomyces cerevisiae and its effect on product formation during anaerobic fermentation" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 65, no. 6, June 1999 (1999-06), pages 2333-2340, XP000856677 the whole document page 334, left-hand column, line 10-20 --- -/--	1-12, 14-16, 18,19, 28-30, 37,38, 41,42, 44-62, 64-68,70

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int: ional Application No
PCT/DK 99/00398

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 28480 A (GENECOR INTERNATIONAL INC ;DU PONT (US); NAIR RAMESH V (US); PAYNE) 10 June 1999 (1999-06-10) the whole document examples 1-10 claims 1-10	1-9,19, 20,25, 27-30, 37-40, 42, 44-61, 65-68,70
P,X	WO 98 54337 A (HANSENS LAB ;NILSSON DAN (DK); KRINGELUM BOERGE (DK)) 3 December 1998 (1998-12-03) abstract page 10, line 2 -page 12, line 7 claims 14-16,30-32,37-41	1-6, 8-11, 28-30, 37,39, 43-45, 47-49, 51,53, 54, 56-58, 63,65, 66,70
P,X	LOPEZ DE FELIPE F ET AL.: "Cofactor engineering: a novel approach to metabolic engineering in Lactococcus lactis by controlled expression of NADH oxidase" JOURNAL OF BACTERIOLOGY, vol. 180, no. 15, August 1998 (1998-08), pages 3804-3808, XP002128387 cited in the application abstract	1-6, 8-11,19, 28-30, 37,39, 43-45, 47-49, 51,53, 54, 56-58, 63,65, 66,70
E	WO 99 46363 A (ARISTIDOU ARISTOS ;VALTION TEKNILLINEN (FI); RICHARD PETER (FI); T) 16 September 1999 (1999-09-16) the whole document examples 1,8,9,13,14	1-11,19, 20,23, 28-30, 37,38, 41,42, 44-62, 65-68,70

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

PATENT CLAIMS

1. Eukaryotic microbial cell comprising an intracellular transhydrogenase activity encoded by a nucleic acid operably linked to an expression signal not natively associated with the nucleic acid, wherein the expression of the intracellular transhydrogenase activity is operably linked to an increased production of a first metabolite.

2. Microbial cell according to claim 1 wherein the expression of the intracellular transhydrogenase activity is further operably linked to a decreased production of the second metabolite.

3. Microbial cell according to claim 1, said cell being selected from the group consisting of a fungal cell and a yeast cell.

4. Microbial cell according to claim 1, said cell being a yeast cell.

5. Microbial cell according to claim 1 wherein the expression of said intracellular transhydrogenase results in an increased level of at least one intracellular coenzyme in its oxidised or reduced form.

6. Microbial cell according to claim 1 wherein said coenzyme in its oxidised/reduced form is NAD/NADH.

7. Microbial cell according to claim 1 wherein said coenzyme in its oxidised/reduced form is NADP/NADPH.

8. Microbial cell according to claim 1 wherein said coenzyme in its oxidised/reduced form is FAD/$FADH_2$.

9. Microbial cell according to claim 1 wherein the expression of said intracellular transhydrogenase results in an increased level of at least one intracellular redox system.

10. Microbial cell according to claim 9 wherein said redox system is NAD/NADH.

11. Microbial cell according to claim 9 wherein said redox system is NADP/NADPH.

12. Microbial cell according to claim 9 wherein said redox system is FAD/$FADH_2$

13. Microbial cell according to claim 1 wherein said intracellular transhydrogenase is a pyridine nucleotide transhydrogenase activity.

14. Microbial cell according to claim 13 wherein said transhydrogenase activity is that encoded by *CTH* of *Azotobacter vinelandii* as harboured by *Saccharomyces cerevisiae* TN4 deposited under DSM Accession Number 12267, or a functionally equivalent activity capable of catalysing a transhydrogenase activity.

15. Microbial cell according to claim 13 wherein said pyridine nucleotide transhydrogenase activity is heterologous to said cell.

16. Microbial cell according to claim 1 wherein said cell harbours no endogenous transhydrogenase activity.

17. Microbial cell according to claim 14 or 15 wherein said transhydrogenase activity is exhibited by a polypeptide which is membrane-bound in a natural host organism.

18. Microbial cell according to claim 14 or 15 wherein said transhydrogenase activity is exhibited by a polypeptide which is located in a cytoplasm of a natural host organism.

19. Microbial cell according to any of the previous claims in the form of a frozen or freeze-dried preparation such as a reconstitutable lyophilisate.

20. Composition comprising the microbial cell according to any of the previous claims and a physiologically acceptable carrier.

21. Composition according to claim 20 wherein said carrier is a water-based liquid and preferably a broth suitable for culturing said microbial cell.

22. Composition according to claims 20 or 21, said composition being a fermentation starter culture.

23. Nucleotide sequence encoding a transhydrogenase enzyme activity, said sequence comprising SEQ ID NO:1, or part thereof, including functionally equivalent derivatives.

24. Nucleotide sequence according to claim 23 wherein the functionally equivalent derivatives comprise conservative nucleotide substitutions and/or nucleotide deletions and/or nucleotide insertions.

25. Recombinant DNA-replicon in the form of a vector comprising the nucleotide sequence according to claim 23 or 24.

26. Recombinant DNA-replicon according to claim 25, said replicon being harboured by *Saccharomyces cerevisiae* TN4 deposited under DSM Accession Number 12267.

27. Microbial cell according to any of claims 1 to 19 transformed with the nucleotide sequence of claim 23 to 24 or the vector of claim 25 or 26.

28. Amino acid sequence encoded by the nucleotide sequence of claim 23 or 24, said sequence comprising SEQ ID NO:2, or part thereof, including functionally equivalent derivatives.

29. Microbial cell according to any of claims 1 to 19 and 27 or composition according to any of claims 20 to 22 for use in a production of a first metabolite.

30. Microbial cell according to claim 29, said cell being a yeast cell.

31. Microbial cell according to claim 30, said cell being a *Saccharomyces cerevisiae* cell.

32. Microbial cell according to claim 29, said cell being selected from the group consisting of a *Penicillium* cell and an *Aspergillus* cell.

33. Microbial cell according to claim 29 wherein said first metabolite is ethanol.

34. Microbial cell according to claim 29 wherein said first metabolite is glycerol.

35. Microbial cell according to any of claims 1 to 19 and 27 or composition according to any of claims 20 to 22 for use in a production of a first metabolite and a second metabolite.

36. Microbial cell according to claim 35 for use in an increased production of a first metabolite and a decreased production of a second metabolite.

37. Microbial cell according to claim 36 wherein said first metabolite is glycerol and said second metabolite is ethanol.

38. Microbial cell according to any of claims 1 to 19 and 27, or composition according to any of claims 20 to 22, for use in a preparation of a drinkable or an edible product.

39. Microbial cell according to any of claims 1 to 19 and 27, or composition according to any of claims 20 to 22, for use in a production of a first metabolite for use in a drinkable or an edible product.

40. Microbial cell according to claim 39, wherein said first metabolite has and/or provides a desirable organoleptic quality to said product.

41. Microbial cell according to claim 39, said first metabolite being selected from the group consisting of ethanol and glycerol.

42. Use of the microbial cell according to any of claims 1 to 19 and 27, or composition according to any of claims 20 to 22, in the production of a first metabolite.

43. Use according to claim 42 wherein said first metabolite is selected from the group consisting of ethanol and glycerol.

44. Use of the microbial cell according to any of claims 1 to 19 and 27, or composition according to any of claims 20 to 22, in the production of a first and a second metabolite.

45. Use according to claim 44 in an increased production of a first metabolite and a decreased production of a second metabolite.

46. Use according to claim 45 wherein said first metabolite is glycerol and said second metabolite is ethanol.

47. Use of a microbial cell according to any of claims 1 to 19 and 27, or composition according to any of claims 20 to 22, in a preparation of a drinkable or edible product.

48. Method of producing a first metabolite comprising the steps of

i) cultivating microbial cell according to any of claims 1 to 19 and 21 in a suitable growth medium and under such conditions that said microbial cell is producing a first metabolite,

and optionally

ii) isolating said first metabolite in a suitable form,

and further optionally

iii) purifying said isolated first metabolite.

49. Method of claim 48 wherein said production of said first metabolite being substantially increased as compared to the production of the metabolite in a comparable wild-type cell or a comparable isolated microbial cell.

50. Method of claim 48 wherein said microbial cell is a yeast cell.

51. Method of claim 48 wherein said first metabolite is glycerol.

52. Method of claim 48, said yeast cell further producing a second metabolite, the production of said second metabolite being substantially decreased as compared to the production of said second metabolite in a comparable wild-type cell or a comparable isolated microbial cell.

53. Method of claim 52 wherein said second metabolite is ethanol.

54. Method of constructing a microbial cell comprising the steps of

i) operably linking a nucleotide sequence encoding a transhydrogenase activity with an expression signal not natively associated with said nucleotide sequence, and

ii) introducing said operably linked nucleotide sequence obtained under i) into a eukaryotic microbial cell wherein said expression signal results in expression of said operably linked nucleotide sequence.

55. Method of claim 54, said microbial cell being selected from the group consisting of a fungal cell and a yeast cell.

56. Method of claim 55, said microbial cell being a yeast cell.

57. Method of claim 56, said yeast cell being a *Saccharomyces cerevisiae* cell.

58. Method of claim 57 wherein said cell is *Saccharomyces cerevisiae* TN4 deposited under DSM Accession Number 12267.

59. Method according to any of claims 54 to 58, said method comprising a further step of freezing or freeze-drying the microbial cell in the preparation of a reconstitutable lyophilisate.

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: NIELSEN 5

U.S. APPLICATION NO. (If known,see 37 CFR 1.5):

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY CLAIMED
PCT/DK99/00398	12 July 1999	10 July 1998

TITLE OF INVENTION: METABOLICALLY ENGINEERED MICROBIAL CELL COMPRISING A MODIFIED REDOX ACTIVITY

APPLICANT(S) FOR DO/EO/US: **Jens NIELSEN et al.**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] The US has been elected in a Demand by the expiration of 19 months from the priority date (PCT Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not transmitted by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An Assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [X] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:
    [X] Courtesy copy of the International Application as filed.
    [X] Courtesy copy of the first page of the International Publication (WO 00/03021).
    [X] **Courtesy copy of the International Preliminary Examination Report with annexes containing claims 1-59 to be substituted for the original claims for examination in this case.**
    [X] Formal drawings, 6 sheets, Figures 1-5.
    [X] Courtesy Copy of the International Search Report.
    [X] Sequence Listing

U.S. APPLICATION NO. (If known,see 37 CFR 1.5)	International Application No. PCT/DK99/00398	Attorney's Docket No. NIELSEN 5

17. [xx] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a)(1) –(5):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....**$1000.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....**$860.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....**$710.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4).....**$690.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....**$100.00**

	CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims as Originally Presented	Number Filed	Number Extra	Rate		
Total Claims	1 - 20		X $18.00	$	
Independent Claims	1 - 3		X $80.00	$	
Multiple Dependent Claims (if applicable)			+$270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 990.00	

Claims After Post Filing Prel. Amend	Number Filed	Number Extra	Rate		
Total Claims	20 - 20		X $18.00	$	
Independent Claims	1 - 3		X $78.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 990.00	

Reduction of ½ for filing by small entity, if applicable. Applicant claims small entity status. See 37 CFR 1.27.	$	
SUBTOTAL =	$ 990.00	
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	$	
TOTAL NATIONAL FEE =	$ 990.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +	$	
TOTAL FEES ENCLOSED =	$ 990.00	
	Amount to be: refunded	$
	charged	$

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.

b. [X] Credit Card Payment Form (PTO-2038), authorizing payment in the amount of $ 990.00, is attached.

c. [ ] Please charge my Deposit Account No. **02-4035** in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. [XX] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **02-4035**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

BROWDY AND NEIMARK, P.L.L.C.
624 NINTH STREET, N.W., SUITE 300
WASHINGTON, D.C. 20001
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Date of this submission: **January 10, 2001**

SIGNATURE

Iver P. Cooper
NAME

28,005
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Jens NIELSEN et al.	)	Art Unit:
IA No.: PCT/DK99/00398	)	
IA Filed: 12 July 1999	)	Washington, D.C.
U.S. App. No.: (Not Yet Assigned)	)	
National Filing Date: (Not Yet Received)	)	January 10, 2001
For: METABLICALLY ENGINEERED...	)	Docket No.: NIELSEN 5

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE SPECIFICATION

After the title please insert the following paragraph:

—The present application is the national stage under 35 U.S.C. 371 of PCT/DK99/00398, filed 12 July 1999.--

IN THE CLAIMS

Delete claims 2-59.

REMARKS

The above amendment to the specification is being made to insert reference to the PCT application of which the present case is a U.S. national stage. The above amendments to the claims are being made in order to eliminate claims, for the purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By: _____
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Jens NIELSEN et al.	)	Art Unit:
IA No.: PCT/DK99/00398	)	
IA Filed: 12 July 1999	)	Washington, D.C.
U.S. App. No.: (Not Yet Assigned)	)	
National Filing Date: (Not Yet Received)	)	January 10, 2001
For: METABLICALLY ENGINEERED...	)	Docket No.: NIELSEN 5

SUPPLEMENTAL PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination upon the merits, kindly amend as follows:

Delete claim 1, and insert the following claims:

—60. Eukaryotic microbial cell comprising an intracellular transhydrogenase activity encoded by a nucleic acid operably linked to an expression signal not natively associated with the nucleic acid,

wherein the intracellular transhydrogenase is a cytoplasmic transhydrogenase when the eukaryotic microbial cell is a yeast cell, and

wherein the expression of the intracellular transhydrogenase activity results in
an increased production of a first metabolite.

61. Microbial cell according to claim 60 wherein the expression of the intracellular transhydrogenase activity further results in a decreased production of the second metabolite.

62. Microbial cell according to claim 60, said cell being selected from the group consisting of a fungal cell and a yeast cell.

63. Microbial cell according to claim 60, said cell being a yeast cell.

64. Microbial cell according to claim 60 wherein the expression of said intracellular transhydrogenase results in an increased level of at least one intracellular coenzyme in its oxidised or reduced form.

65. Microbial cell according to claim 64 wherein said coenzyme in its oxidised/reduced form is NAD/NADH.

66. Microbial cell according to claim 64 wherein said coenzyme in its oxidised/reduced form is NADP/NADPH.

67. Microbial cell according to claim 64 wherein said coenzyme in its oxidised/reduced form is FAD/$FADH_2$.

68. Microbial cell according to claim 60 wherein said intracellular transhydrogenase is a pyridine nucleotide transhydrogenase.

69. Microbial cell according to claim 68 wherein said transhydrogenase activity is that encoded by CTH of Azotobacter vinelandii as harboured by Saccharomyces

cerevisiae TN4 deposited under DSM Accession Number 12267, or a function ally equivalent activity capable of catalysing a transhydrogenase.

70. Microbial cell according to claim 68 wherein said pyridine nucleotide trans 10 hydrogenase is heterologous to said cell.

71. Microbial cell according to claim 68 wherein said cell harbours no endogenous transhydrogenase.

72. Composition comprising the microbial cell according to claim 60 and a physiologically acceptable carrier.

73. Microbial cell according to claim 60 wherein said first metabolite is ethanol.

74. Microbial cell according to claim 60 wherein said first metabolite is glycerol.

75. Microbial cell according to claim 60 for use in a preparation of a drinkable or an edible product.

76. Method of producing a first metabolite comprising the steps of

i) cultivating microbial cell according to claim 60 in a suitable growth medium and under such conditions that said microbial cell is producing a first metabolite, 30

and optionally

ii) isolating said first metabolite in a suitable form,

and further optionally purifying said isolated first metabolite.

77. Method of claim 76, said yeast cell further producing a second metabolite, the production of said second metabolite being substantially decreased as com pared to the production of said second metabolite in a comparable wild-type cell or a comparable isolated microbial cell.

78. Method of claim 77 wherein said first metabolite is ethanol and said second metabolite is glycerol.

79. Method of constructing a microbial cell according to claim 60 comprising the steps of

i) operably linking a nucleotide sequence encoding a transhydrogenase activity with an expression signal not natively associated with said nucleotide sequence, and

ii) introducing said operably linked nucleotide sequence obtained under i) into a eukaryotic microbial cell, wherein said expression signal results in expression of said operably linked nucleotide sequence

wherein the intracellular transhydrogenase is a cytoplasmic transhydrogenase when the eukaryotic microbial cell is a yeast cell.—

REMARKS

Claims 60-79 presently appear in this case. The above amendments to the claims are being made in order to add new claims and to restore at least partly the varying scope of claims which was eliminated by the elimination of multiple dependencies in the claims.

Favorable consideration is earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
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